Use of NCCN Guidelines, Other Guidelines, and Biomarkers for Colorectal Cancer Screening

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Abstract

Colorectal cancer (CRC) remains a common cancer and significant public health burden. CRC-related mortality is declining, partly due to the early detection of CRC through robust screening. NCCN has established the NCCN Guidelines for CRC Screening to help healthcare providers make appropriate screening recommendations according to the patient's risk of developing CRC. This review describes the evolution of CRC screening guidelines for average-risk individuals, discusses the role of NCCN Guidelines for CRC Screening in cancer prevention, and comments on the current and emerging use of biomarkers for CRC screening.

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Background

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in the United States and the second leading cause of cancer-related death among men and women. In 2016, an estimated 134,490 new CRC cases and 49,190 CRC-related deaths are expected.¹ Despite the considerable public health burden of CRC, incidence and mortality rates have significantly declined in the past few decades; this trend is mainly attributed to treatment innovations and increased CRC screening.^{1–3} Among average-risk individuals, the goals of CRC screening are 2-fold: (1) identify and remove precancerous polyps, thereby reducing CRC incidence, and (2) detect CRC at an early stage when curative therapy is most likely possible, thereby reducing CRC mortality.

Currently, 40% of CRC cases are diagnosed as localized disease,³ with a 5-year survival rate of 90%.⁴ This review describes the evolution of CRC screening guidelines for average-risk individuals, identifies predictors of adherence to these screening guidelines, discusses the role of the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for CRC Screening in cancer prevention, and comments on the current and emerging use of biomarkers for CRC screening (to view the most recent version of these guidelines, visit NCCN.org).

Evolution and Comparison of CRC Screening Guidelines

Over the past 30 years, guidelines and available options for CRC screening have evolved. In the 1980s,

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for average-risk individuals aged 50 years or older, formal CRC screening guidelines focused on annual guaiac fecal occult blood test (gFOBT). Since that time, professional societies have revised their guidelines regarding the use of fecal immunochemical tests (FIT), sigmoidoscopy, barium enema, and colonoscopy, and their use as appropriate screening modalities. Recently, FIT-DNA (ie, FIT plus stool DNA) and CT colonography (CTC) have also been included as treatment modalities.⁵

The most sensitive screening tests for reducing CRC mortality have the ability to detect advanced serrated polyps, advanced adenomatous polyps, and cancer. Colonoscopy is one such test and, thus, may be preferred over other screening modalities that can detect cancer but not polyps (ie, stool-based tests). Because of its ability to detect and remove precancerous polyps, colonoscopy is a useful and unique tool not only in the early detection of cancer but also in cancer prevention.⁶

A number of organizations have established recommendations and clinical practice guidelines for CRC screening, which encompass multiple screening modalities. CRC screening guidelines have been developed by the US Preventive Services Task Force (USPSTF),⁷ American Cancer Society,⁸ American College of Gastroenterology,⁹ U.S. Multi-Society Task Force,¹⁰ and NCCN,⁴ among others. Although there is general consistency among the recommendations set forth by these organizations, there are also a few notable differences in guidance. Table 1 illustrates how the NCCN Guidelines for CRC

Screening compare with those of other professional societies.

The NCCN Guidelines for CRC Screening were established to help providers make appropriate CRC screening recommendations based on patients' risk of developing CRC.⁴ Recommended screening methods, age at which to initiate screening, and frequency of screening vary by individual risk level. For high-risk patients (ie, those with a personal history of adenomatous polyps, sessile serrated polyps, CRC, inflammatory bowel disease, or family history), the only screening method recommended is colonoscopy.⁴ In contrast, for average-risk patients, NCCN recommends a choice of initial screening methods, such as colonoscopy, stool-based test, or flexible sigmoidoscopy with or without a stool-based test.⁴ NCCN does not currently recommend screening with CTC or barium enema.⁴ Similarly, the USPSTF guidelines do not include barium enema due to the availability of more sensitive tests. Additionally, recommendations regarding the use of flexible sigmoidoscopy vary in 2 ways: (1) whether gFOBT and/or FIT are included, and (2) the suggested screening interval.

Recently, both USPSTF and NCCN revised aspects of their recommendations regarding CRC screening based on recently published evidence.^{2,11} The crux of the updated USPSTF recommendations is the absence of a preferred screening method and added emphasis on evidence that CRC screening, in general, reduces CRC mortality for asymptomatic individuals aged 50 to 75 years.^{12,13} The updated evidence review did not purport that one screening

Table 1. Summary of Colorectal Cancer Screening Guidelines

	NCCN ²	USPSTF ¹¹	ACS/USMSTF/ACR ⁶	ACG ⁹
Detect cancer				
gFOBT	Annual	Annual	Annual	Annual
FIT	Annual	Annual	Annual	Annual
Stool DNA (FIT-DNA)	Every 3 y	Every 1 or 3 y	Every 3 y	Interval uncertain
Detect cancer and polyps				
Flexible sigmoidoscopy	Every 5–10 y ± gFOBT/FIT at y 3	Every 5 y OR Every 10 y + annual FIT	Every 5 y ± annual gFOBT/FIT	Every 5 y
Colonoscopy	Every 10 y	Every 10 y	Every 10 y	Every 10 y
CTC	–	–	Every 5 y	Every 5 y
Barium enema	–	–	Every 5 y	Every 5 y

Abbreviations: ACG, American College of Gastroenterology; ACR, American College of Radiology; ACS, American Cancer Society; CTC, CT colonography; FIT, fecal immunochemical test; gFOBT, guaiac fecal occult blood test; USMSTF, U.S. Multi-Society Task Force; USPSTF, US Preventive Services Task Force.

method was superior to another as it relates to the net benefit.⁷ The current list of screening options now includes FIT-DNA every 1 or 3 years, CTC every 5 years, and flexible sigmoidoscopy every 5 years or every 10 years with annual FIT. The USPSTF's current guidance further highlights that although this mortality benefit is greatest for those aged 50 to 75 years, the benefit for older patients is minimal. Therefore, the decision to screen should take into consideration the health and screening history for those aged 76 to 85 years, with no screening recommended for those older than 85 years. Because screening rates are suboptimal, with at least one-third of eligible patients having never been screened, the USPSTF asserts that the "best screening test is the one that gets done." Shared decision-making is an approach to choosing the "best" and preferred test for each patient, and it has been shown that patients are more likely to adhere to screening when given a choice of screening method.¹⁴

A key modification to NCCN Guidelines for average-risk individuals is the emphasis that screening not only lowers CRC mortality, but also reduces incidence by identifying and removing polyps.² Previously, the recommended interval for flexible sigmoidoscopy was every 5 years with or without stool-based testing every 3 years, whereas current recommendations call for flexible sigmoidoscopy every 5 to 10 years with or without gFOBT/FIT at year 3. NCCN Guidelines now recommend the option of stool DNA for CRC detection with a suggested interval of 3 years, although the appropriateness of this interval is uncertain.¹⁵ Regarding stool-based screening in general, the guidelines note that evidence showing FIT is more sensitive than gFOBT was based solely on nonrandomized studies, which also indicate lower mortality with FIT. Language referencing colonoscopy as the "primary method" for CRC screening was modified to "most common method," presumably to remove the suggestion that colonoscopy is preferred over other screening modalities.

Rates and Predictors of Adherence to Guidelines for Initial CRC Screening

Given the National Colorectal Cancer Roundtable's goal to achieve CRC screening compliance rates of 80% by 2018, it is critical to assess current rates and identify predictors of screening adherence.¹⁶ Studies

have shown that older patients and, to a lesser extent, those who have a higher comorbidity burden,¹⁷ are uninsured,¹⁸ lack a high school education, are of certain race/ethnic groups, and report certain health beliefs are less likely to undergo CRC screening.¹⁹

A recent 10-year longitudinal study evaluated adherence to USPSTF guidelines and determined that only 64% of insured patients in their cohort were screened in accordance with the guidelines. Of those screened, screening was initiated an average of 3 years later than recommended.²⁰ A separate study at a Veterans Affairs (VA) medical center evaluated the impact of a national VA CRC screening initiative by comparing screening rates and outcomes before and after the 1998 initiative.²¹ The screening rates before and after implementation of the initiative were 45% and 50%, respectively. Pre- and post-initiative stage distribution was only suggestive of a trend toward early-stage diagnoses. However, when considering anatomic location, there was a significant shift toward early-stage diagnoses among distal cancers after the initiative, but no change in stage distribution for proximal cancers. Among all patients with screen-detected CRC, the 5-year all-cause survival rate was approximately 89%.

In 2010, the prevalence estimate of screening by either FOBT or endoscopy (ie, sigmoidoscopy or colonoscopy) was 59%.³ Screening rates vary by a number of demographic and socioeconomic factors, including patient age, race, years of education, insurance status, and immigration status.³ In a study by Shapiro et al²² using National Health Interview Survey data, the prevalence of guideline-concordant self-reported CRC screening by any means was 58%, with the use of colonoscopy being 55%. Substantial differences in receipt of screening were noted within categories of income, education, type of insurance, and source of healthcare. The study authors also captured the patient reasons for deciding not to undergo CRC screening, with the most commonly reported reason being "no reason or never thought about it" (41%), followed by a lack of provider recommendation (15%) and absence of symptoms (14%).²² A retrospective cohort study of screening-eligible patients in a safety-net health system reported 22% of patients being screened in the prior 5 years, with significant predictors of screening participation being female sex, Hispanic ethnicity, age of 65 to 75 years, and having access to care.²³ Marital status is

another important predictor of screening, according to a large study of approximately 300,000 participants that noted that, compared with nonpartnered people, married and unmarried couples were more likely to undergo CRC screening.²⁴

Current and Emerging Use of Biomarkers for CRC Screening

Despite a strong evidence base supporting its efficacy, colonoscopy is limited by its invasiveness, expense, and suboptimal patient compliance, whereas FOBT and FIT have low sensitivities for detecting colon polyps. Thus, efforts are underway to develop more sensitive and specific noninvasive biomarker assays as another means of risk stratification and early detection of advanced colon polyps or CRC. As part of the colorectal carcinogenesis process, gene mutations and epigenetic alterations arise in colon polyps and CRCs, and are potentially highly specific diagnostic biomarkers for the detection of colonic polyps and cancers. Feces and blood are the analytes used in the best developed biomarkers for CRC screening at this time, and they are the analytes used in the recently FDA-approved CRC biomarkers.

Fecal-Based Biomarkers

Since the discovery of mutant *KRAS* in fecal specimens of patients with CRC,²⁵ numerous studies have supported using fecal DNA for potential screening assays for early CRC detection.^{26–28} To date, biomarkers assessed include mutant genes, methylated genes, microRNAs, and mRNA. However, to date, the most successful biomarkers are based on methylated DNA.

Many phase I and II biomarker studies have identified fecal-based methylation biomarkers for the early detection of CRC.²⁹ Studies of methylated *SFRP2*, *SFRP5*, *PGR*, *CALCA*, and *IGFBP2* in fecal DNA identified methylated *SFRP2* as a diagnostic biomarker for CRC detection with high sensitivity (77%–90%) and specificity (77%).³⁰ A second study of stool DNA markers among patients with colorectal adenomas demonstrated that methylated *SFRP2* can also identify patients with precancerous colonic polyps.³¹ Another well-studied fecal DNA biomarker for CRC detection is methylated *VIM*, the gene for vimentin. Methylated *VIM* specifically occurs in adenoma and CRC tissues, and is detected in the fecal DNA of patients with colon neoplasms

with reasonably high sensitivity (46%) and specificity (90%).³² Studies demonstrating the potential of using methylated *VIM* as a biomarker for the early detection of CRC has led to the development of an assay that detects methylated *VIM* and is one of the first commercial fecal-DNA screening tests for CRC (ColoSure, Lab Corp, Burlington, NC). Other hypermethylated genes (*APC*, *ATM*, *BMP3*, *CDKN2A*, *SFRP2*, *GATA4*, *GSTP1*, *HLTF*, *MLH1*, *MGMT*, *NDRG4*, *RASSF2A*, *TFPI2*, *VIM*, and *WIF1*) have been analyzed in fecal DNA for the early detection of CRC.^{26,32–40}

Efforts to develop an accurate noninvasive biomarker assay for CRC and colon polyp detection recently culminated in the development of Cologuard (Exact Sciences Corporation, Madison, WI), an FDA-approved, clinically available stool-based CRC screening test that combines both the stool DNA test and the OC-Auto FIT (Poly-medco CDP, LLC, Cortlandt Manor, NY). This stool DNA–based assay, which detects methylated *BMP3*, methylated *NDRG4*, and mutant *KRAS*, was recently compared with FIT and demonstrated better sensitivity than FIT for both CRC and advanced adenomas, but lower specificity for both end points.^{27,41} The Cologuard assay is approved for use by the FDA and in the European Union; however, its appropriate use in clinical care is in evolution. Of note, the Cologuard assay has been included as a screening method in the 2016 USPSTF screening guidelines and the 2014 American Cancer Society's CRC prevention and early detection guidelines.

Blood-Based Biomarkers

Because of accessibility and high patient acceptance, blood is invariably the most ideal analyte for cancer biomarkers. With the development of highly sensitive detection methods, such as massively parallel sequencing, there have been renewed efforts to determine whether blood-based diagnostic assays based on circulating methylated DNA can be used for detection of colon polyps or CRC. Okugawa et al²⁶ recently summarized aberrantly methylated genes discovered in the plasma or serum of patients with CRC which, consequently, are candidate biomarkers.

After initial reports of methylated *CDKN2A* in circulating DNA of patients with a variety of cancers in 1999,^{42–44} a growing number of studies have examined the potential of methylated genes to be

blood-based biomarkers for patients with CRC.^{26,27} Currently, the most established methylated DNA blood biomarker is methylated septin 9 (*SEPT9*). Lofton-Day et al⁴⁵ identified methylated *SEPT9* as a noninvasive diagnostic biomarker for CRC with 69% sensitivity and 86% specificity for distinguishing patients with CRC from healthy individuals. Upon further refinement and validation of assays that use methylated *SEPT9* as a biomarker for CRC screening, it is now offered commercially as a blood-based screening test in various assays including Epi proColon 1.0 (Epigenomics, Seattle, WA), ColoVantage (Quest Diagnostics, Madison, NJ) and RealTime mS9 (Abbott Laboratories, Des Plaines, IL). A recent prospective clinical trial (PRESEPT) demonstrated equivalent sensitivity and specificity of methylated *SEPT9* versus FOBT for CRC, confirming its potential usefulness as a blood-based biomarker for CRC.⁴⁶ This test was recently approved by the FDA for use as a CRC screening assay. However, methylated *SEPT9* has a limited sensitivity for the detection of advanced adenomas (11%), underscoring the need for further improvement of this test for implementation for population-based screening of colorectal neoplasia. A recent study demonstrated that the methylated *SEPT9* assay was superior to FIT at detecting CRC, but both approaches were suboptimal for diagnosing patients with advanced adenomas.⁴⁷ To date, several other blood-based diagnostic methylation biomarkers have been identified for CRC detection, including *ALX4*,⁴⁸ *APC*,³⁵ *CDKN2A*,³⁹ *HLTF*,⁴⁹ *HPPI*,⁵⁰ *hMLH1*,⁴⁹ *MGMT*,³⁵ *NEUROG1*,⁵¹ *NGFR*, *RASS-F2A*,³⁵ *SFRP2*, *VIM*,⁵² and *WIF1*.³⁵ Unfortunately, there are no phase III biomarker studies of mutant or methylated DNA-based biomarker assays for CRC detection in a screening setting at this time.⁵³ Furthermore, and importantly, when considered in the context of other CRC and colon polyp screening methods, although a robust biomarker panel of methylated genes may be developed into a clinically accurate CRC screening method in the near future, the currently available blood-based biomarker assays are not appropriate as colon polyp detection methods.

Future Directions

Given the consensus that CRC screening is beneficial yet underutilized, efforts to continue to improve screening rates will expand and will likely focus on

historically underscreened populations. Therefore, having a clear, and ideally unified, set of guidelines could assist in this endeavor. These unified guidelines should be accompanied by research documenting screening adherence, improvements in health outcomes, and whether screening capacity is adequate to achieve current benchmarks. Despite their limitations, blood-based tests have the potential to increase screening rates because they are noninvasive. Moreover, blood-based tests are relatively simple to conduct and may be more acceptable to patients than more invasive and labor-intensive options. Additionally, shared decision-making could result in choosing a screening option that is most consistent with patients' values and preferences, thus improving adherence for initial and, if necessary, follow-up testing. Additional interventions to improve initial CRC screening may include screening programs targeting screening-naïve and historically underscreened populations, improved care coordination, and use of patient and provider reminder tools. As CRC screening recommendations continue to evolve and new technologies and biomarkers emerge, there may be heightened interest in measuring adherence to clinical practice guidelines as a quality indicator. Systematic monitoring of screening and associated health outcomes must be developed and implemented.

Conclusions

Although CRC screening rates have increased dramatically over the past few decades, they remain suboptimal. Reasons for underutilization of CRC screening methods are multifactorial and accordingly require changes at multiple levels, including the patient, provider, and healthcare system. The guidelines developed by numerous professional organizations continue to evolve as more evidence becomes available regarding the sensitivity, specificity, benefits, harms, cost, and availability of current and emerging screening options. There is variation between guidelines regarding which screening methods are endorsed and the associated time intervals for screening; the screening intervals for FIT-DNA and flexible sigmoidoscopy are the least consistent across guidelines. The revised guidelines should focus less on the specific screening methods provided to patients and instead emphasize the importance of increasing CRC screening rates overall. Although unified guidance for CRC

screening may not be on the immediate horizon, improving uptake of CRC screening and, consequently, improving early detection and reducing mortality is something that all societies support.

References

1. American Cancer Society. Cancer Facts & Figures 2016. Available at: <http://www.cancer.org/acs/groups/content/@research/documents/document/acspc-047079.pdf>. Accessed July 28, 2016.
2. Provenzale D, Gupta S, Ahnen DJ, et al. Colorectal Cancer Screening, version 1.2016. National Comprehensive Cancer Network. Accessed July 27, 2016. To view the most recent version of these guidelines, visit NCCN.org.
3. Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin* 2014;64:104–117.
4. Provenzale D, Jasperson K, Ahnen DJ, et al. Colorectal Cancer Screening, version 1.2015. *J Natl Compr Canc Netw* 2015;13:959–968; quiz 968.
5. Jensen CD, Corley DA, Quinn VP, et al. Fecal immunochemical test program performance over 4 rounds of annual screening: a retrospective cohort study. *Ann Intern Med* 2016;164:456–463.
6. Levin B, Lieberman DA, McFarland B, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *Gastroenterology* 2008;134:1570–1595.
7. Whitlock EP, Lin JS, Liles E, et al. Screening for colorectal cancer: a targeted, updated systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2008;149:638–658.
8. Byers T, Levin B, Rothenberger D, et al. American Cancer Society guidelines for screening and surveillance for early detection of colorectal polyps and cancer: update 1997. American Cancer Society Detection and Treatment Advisory Group on Colorectal Cancer. *CA Cancer J Clin* 1997;47:154–160.
9. Rex DK, Johnson DA, Anderson JC, et al. American College of Gastroenterology guidelines for colorectal cancer screening 2008. *Am J Gastroenterol* 2009;104:739–750.
10. Lieberman DA, Rex DK, Winawer SJ, et al. Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology* 2012;143:844–857.
11. Lin JS, Piper MA, Perdue LA, et al. Screening for colorectal cancer: updated evidence report and systematic review for the US Preventive Services Task Force. *JAMA* 2016;315:2576–2594.
12. Bibbins-Domingo K, Grossman DC, Curry SJ, et al. Screening for colorectal cancer: US Preventive Services Task Force recommendation statement. *JAMA* 2016;315:2564–2575.
13. Ransohoff DF, Sox HC. Clinical practice guidelines for colorectal cancer screening: new recommendations and new challenges. *JAMA* 2016;315:2529–2531.
14. Inadomi JM, Vijan S, Janz NK, et al. Adherence to colorectal cancer screening: a randomized clinical trial of competing strategies. *Arch Intern Med* 2012;172:575–582.
15. Berger BM, Parton MA, Levin B. USPSTF colorectal cancer screening guidelines: an extended look at multi-year interval testing. *Am J Manag Care* 2016;22:e77–81.
16. Meester RG, Doubeni CA, Zuber AG, et al. Public health impact of achieving 80% colorectal cancer screening rates in the United States by 2018. *Cancer* 2015;121:2281–2285.
17. Klabunde CN, Zheng Y, Quinn VP, et al. Influence of age and comorbidity on colorectal cancer screening in the elderly. *Am J Prev Med* 2016;51:e67–75.
18. Joseph DA, King JB, Miller JW, Richardson LC. Prevalence of colorectal cancer screening among adults—behavioral risk factor surveillance system, United States, 2010. *MMWR Suppl* 2012;61:51–56.
19. Stanley SL, King JB, Thomas CC, Richardson LC. Factors associated with never being screened for colorectal cancer. *J Community Health* 2013;38:31–39.
20. Cyhaniuk A, Coombes ME. Longitudinal adherence to colorectal cancer screening guidelines. *Am J Manag Care* 2016;22:105–111.
21. van Roessel P, Rouse RV, Wren SM. Care within a Veterans Hospital – earlier detection of colon cancer. *Surg Endosc* 2007;21:1434–1440.
22. Shapiro JA, Klabunde CN, Thompson TD, et al. Patterns of colorectal cancer test use, including CT colonography, in the 2010 National Health Interview Survey. *Cancer Epidemiol Biomarkers Prev* 2012;21:895–904.
23. Gupta S, Tong LY, Allison JE, et al. Screening for colorectal cancer in a safety-net health care system: access to care is critical and has implications for screening policy. *Cancer Epidemiol Biomarkers Prev* 2009;18:2373–2379.
24. El-Haddad B, Dong F, Kallail KJ, et al. Association of marital status and colorectal cancer screening participation in the USA. *Colorectal Dis* 2015;17:O108–114.
25. Sidransky D, Tokino T, Hamilton SR, et al. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. *Science* 1992;256:102–105.
26. Okugawa Y, Grady WM, Goel A. Epigenetic alterations in colorectal cancer: emerging biomarkers. *Gastroenterology* 2015;149:1204–1225.
27. Dickinson BT, Kiesel J, Ahlquist DA, Grady WM. Molecular markers for colorectal cancer screening. *Gut* 2015;64:1485–1494.
28. Shah R, Jones E, Vidart V, et al. Biomarkers for early detection of colorectal cancer and polyps: systematic review. *Cancer Epidemiol Biomarkers Prev* 2014;23:1712–1728.
29. Pepe MS, Alonzo TA. Comparing disease screening tests when true disease status is ascertained only for screen positives. *Biostatistics* 2001;2:249–260.
30. Muller HM, Oberwalder M, Fiegl H, et al. Methylation changes in faecal DNA: a marker for colorectal cancer screening? *Lancet* 2004;363:1283–1285.
31. Huang Z, Li L, Wang J. Hypermethylation of SFRP2 as a potential marker for stool-based detection of colorectal cancer and precancerous lesions. *Dig Dis Sci* 2007;52:2287–2291.
32. Chen WD, Han ZJ, Skoletsky J, et al. Detection in fecal DNA of colon cancer-specific methylation of the nonexpressed vimentin gene. *J Natl Cancer Inst* 2005;97:1124–1132.
33. Glockner SC, Dhir M, Yi JM, et al. Methylation of TFP12 in stool DNA: a potential novel biomarker for the detection of colorectal cancer. *Cancer Res* 2009;69:4691–4699.
34. Hellebrekers DM, Lentjes MH, van den Bosch SM, et al. GATA4 and GATA5 are potential tumor suppressors and biomarkers in colorectal cancer. *Clin Cancer Res* 2009;15:3990–3997.
35. Lee BB, Lee EJ, Jung EH, et al. Aberrant methylation of APC, MGMT, RASSF2A, and Wif-1 genes in plasma as a biomarker for early detection of colorectal cancer. *Clin Cancer Res* 2009;15:6185–6191.
36. Leung WK, To KF, Man EP, et al. Detection of hypermethylated DNA or cyclooxygenase-2 messenger RNA in fecal samples of patients with colorectal cancer or polyps. *Am J Gastroenterol* 2007;102:1070–1076.
37. Lidgard GP, Domanico MJ, Bruinsma JJ, et al. Clinical performance of an automated stool DNA assay for detection of colorectal neoplasia. *Clini Gastroenterol Hepatol* 2013;11:1313–1318.
38. Melotte V, Lentjes MH, van den Bosch SM, et al. N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumor suppressor gene and potential biomarker for colorectal cancer. *J Natl Cancer Inst* 2009;101:916–927.
39. Petko Z, Ghiassi M, Shuber A, et al. Aberrantly methylated CDKN2A, MGMT, and MLH1 in colon polyps and in fecal DNA from patients with colorectal polyps. *Clin Cancer Res* 2005;11:1203–1209.
40. Wang DR, Tang D. Hypermethylated SFRP2 gene in fecal DNA is a high potential biomarker for colorectal cancer noninvasive screening. *World J Gastroenterol* 2008;14:524–531.
41. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Multitarget stool DNA testing for colorectal-cancer screening. *N Engl J Med* 2014;370:1287–1297.
42. Wong IH, Lo YM, Zhang J, et al. Detection of aberrant p16 methylation in the plasma and serum of liver cancer patients. *Cancer Res* 1999;59:71–73.
43. Esteller M, Sanchez-Cespedes M, Rosell R, et al. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. *Cancer Res* 1999;59:67–70.
44. Silva JM, Dominguez G, Villanueva MJ, et al. Aberrant DNA methylation of the p16INK4a gene in plasma DNA of breast cancer patients. *Br J Cancer* 1999;80:1262–1264.
45. Lofton-Day C, Model F, Devos T, et al. DNA methylation biomarkers for blood-based colorectal cancer screening. *Clin Chem* 2008;54:414–423.
46. Church TR, Wandell M, Lofton-Day C, et al. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. *Gut* 2014;63:317–325.

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47. Jin P, Kang Q, Wang X, et al. Performance of a second-generation methylated SEPT9 test in detecting colorectal neoplasm. *J Gastroenterol Hepatol* 2015;30:830–833.
 48. Ebert MP, Model F, Mooney S, et al. Aristaless-like homeobox-4 gene methylation is a potential marker for colorectal adenocarcinomas. *Gastroenterology* 2006;131:1418–1430.
 49. Leung WK, To KF, Man EP, et al. Quantitative detection of promoter hypermethylation in multiple genes in the serum of patients with colorectal cancer. *Am J Gastroenterol* 2005;100:2274–2279.
 50. Wallner M, Herbst A, Behrens A, et al. Methylation of serum DNA is an independent prognostic marker in colorectal cancer. *Clin Cancer Res* 2006;12:7347–7352.
 51. Herbst A, Rahmig K, Stieber P, et al. Methylation of NEUROG1 in serum is a sensitive marker for the detection of early colorectal cancer. *Am J Gastroenterol* 2011;106:1110–1118.
 52. Li M, Chen WD, Papadopoulos N, et al. Sensitive digital quantification of DNA methylation in clinical samples. *Nat Biotechnol* 2009;27:858–863.
 53. Mansour H. Cell-free nucleic acids as noninvasive biomarkers for colorectal cancer detection. *Front Genet* 2014;5:182.